(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 October 2001 (11.10.2001)

PCT

(10) International Publication Number
WO 01/75416 A1

(51) International Patent Classification[7]: G01N 1/38

(21) International Application Number: PCT/SE01/00705

(22) International Filing Date: 30 March 2001 (30.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0001175-9 31 March 2000 (31.03.2000) SE

(71) Applicant *(for all designated States except US)*: **BOULE MEDICAL AB** [SE/SE]; Box 42056, S-126 13 Stockholm (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **BERNDTSSON, Ingemar** [SE/SE]; Borevägen 9, S-191 43 Sollentuna (SE).

(74) Agent: **EHRNER & DELMAR PATENTBYRÅ AB**; Box 103 16, S-100 55 Stockholm (SE).

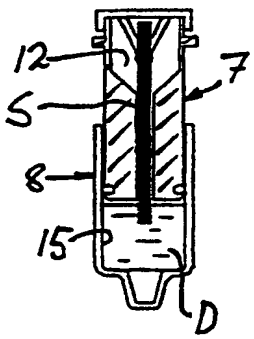
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A DEVICE FOR DILUTING AND MIXING A LIQUID SAMPLE



(57) Abstract: A diluting and mixing device has a piston part (7) and a cylinder part (8). An axial bore (10) through the piston part is adapted to receive a tube (1) containing a liquid sample. An upper portion of the bore forms a first mixing chamber (12). A closed end of the cylinder part (8) defines a receptacle (15) for a diluting liquid (D) and a second mixing chamber. Movement of the piston part (7) within the cylinder part (8) towards its closed end forces diluting liquid (D) from the receptacle (15) through the bore towards the first mixing chamber (12) thereby flushing through a tube (1) positioned in the bore (10) to mix in the first mixing chamber (12) with a sample (S) displaced from the tube. Movement of the piston part (7) away from the closed end causes flow of diluted sample through the bore past the tube towards the second mixing chamber (15).

WO 01/75416 A1

A DEVICE FOR DILUTING AND MIXING A LIQUID SAMPLE

The present invention concerns a device for diluting and mixing a liquid sample, particularly, but not exclusively, a blood sample for performing a CRP test, said device including receiving means for a tubular sample recipient, first receptacle means for a diluting liquid, and mixing chamber means.

Background art includes the Blood Testing Method and Apparatus disclosed in SE-9800214-0 (US Patent Appln. No. 09/239804) by the same inventor.

This prior art method and apparatus uses a capillary tube to contain a defined volume of a blood sample to be tested. In use of the apparatus, a capillary tube containing a blood sample is mounted in an adapter, and the adapter is inserted in a diluting liquid conduit of the apparatus. The adapter is arranged such that flow of diluting liquid through the diluting liquid conduit passes at least partly through the capillary tube, flushes it and brings the sample to a diluting and mixing vessel.

It is an object of the present invention to provide a new device of the general kind initially stated, which is adapted to dilute a sample by mixing it with a diluting agent to provide a diluted sample.

It is also an object of the present invention to further develop this new device to perform a two-stage diluting and mixing operation, viz. a first stage including diluting a sample by mixing it with a diluting agent to provide a diluted sample, and a second step including introduction of a third medium in said diluted sample and mixing said third medium with said diluted sample.

Embodiments of the present invention will now be described, reference being made to the accompanying drawings, wherein:

Fig. 1 is a schematic view of a capillary tube having a combined handle and cover;

Fig. 2 is a schematic view of a first embodiment of a device according to the present invention shown in its preparatory state;

Fig. 3 is a view similar to Fig. 2, showing the cover being removed;

Fig. 4 is a view of the capillary tube collecting a blood sample from a finger tip;

Fig. 5 is a view similar to Fig. 3 showing the capillary tube with blood sample placed in the device;

Fig. 6 is a view similar to Fig. 5 showing a limited downward stroke of the piston part relative to the cylinder part of the device;

Fig. 7 is a view similar to Fig. 6 showing an upward stroke of the piston part relative to the cylinder part;

Fig. 8 is a view similar to Fig. 6 showing an extended downward stroke of the piston part relative to the cylinder part;

Fig. 9 is a view similar to Fig. 7 showing a full upward stroke of the piston part relative to the cylinder part;

Fig. 10 is a part-sectional view of a second embodiment of a device according to the present invention having a

modified combined handle and cover with a capillary tube mounted therein, the device being shown with its piston part and cylinder part in a first relative stroke position;

Fig. 11 is a view corresponding to Fig. 10, but showing the device with its piston part and cylinder part in a second relative stroke position; and

Fig. 12 is an isometric view of an analysis instrument for use with the device of Figs. 10 and 11.

Fig. 1 shows a capillary tube 1 having an upper end 2 and a lower end 3. The tube is mounted in a cover 4 by means of ribs 5 depending from the cover such that the upper end 2 of the tube opens in an open space between the ribs 5 communication with atmosphere.

The first embodiment of a diluting and mixing device 6 according to the present invention shown in Fig. 2 is presented in a simplified state in order to facilitate the understanding of the principles of the invention. The device 6 includes a piston part 7 and a cylinder part 8, the piston part being slidably inserted in the cylinder part. A sealing ring 9 seals between the two parts.

The piston part 7 has a central bore 10 extending axially therethrough. A lower portion 11 of the bore has an inner diameter somewhat larger than the outer diameter of the capillary tube 1, whereas an upper portion 12 thereof is widened and forms a first mixing chamber. A cover 13 having dimensions corresponding to those of the cover 4 closes an upper end of the widened bore portion 12 by sealing against upper free edges 14 of the piston part.

The cylinder part 8 includes a primary cylinder portion 15 being a true straight circular cylinder matching the dimension of the piston part and having a relatively large volume constituting a receptacle means for diluting liquid, and, at the bottom of the primary portion, a secondary space 16 having a relatively small volume. The secondary space is sealed from the primary portion by a diaphragm 17. To achieve the first object of the invention, i.e., to enable just mixing of a sample and a diluting agent, the cylinder part could end with the primary cylinder portion 15, i.e., a bottom wall would be located at the location of the diaphragm 17. Since it is presently preferred, however, to make the cylinder part with its secondary space 16, whether it be used or not, the simple embodiment of the device without the secondary space will not be separately described.

In the preparatory state of the device, the primary cylinder portion 15 contains a first medium such as a predetermined volume (typically 1 ml) of diluting liquid D to about half the cylinder height, and the piston part 7 is introduced in the primary cylinder portion so as to be located with its lower end at or near the level of the diluting liquid D. A second medium, such as a likewise predetermined volume (typically 20 μl) of antibodies A, is contained within the secondary space 16.

In operation of the device, a blood sample S is taken with the capillary tube 1 as illustrated in Fig. 4. The tube is approached to a drop of blood B formed on a punctured finger tip F, and the drop is sucked up by capillary action to completely fill the tube with a defined volume of sample S. During this operation, the cover 4 serves as a handle, thus avoiding any contact with the sample. Now, after having

removed the cover 13 as illustrated in Fig. 3, the capillary tube is inserted into the vertically directed bore 10 through its widened portion 12, and the cover 4 is pushed down to seal against the upper edges 14 thereof as illustrated in Fig. 5. In this position, where the lower end 3 of the capillary tube may (as shown) or may not be submerged in the diluting liquid D, a diluting and mixing step is initiated.

The diluting and mixing step is started by pressing the piston part 7 further into the cylinder part 8 as illustrated in Fig. 6. Hereby, diluting liquid D is forced into and up through the capillary tube, thus displacing the sample contained therein out through its upper end 2 to freely flow down into the widened portion 12 of the bore 10 forming a first mixing chamber. Simultaneously, part of the diluting liquid is forced up through the bore 10, i.e., portions thereof surrounding the capillary tube, and from below into the widened portion 12 of the bore. There it meets the sample and the part of the diluting liquid flushed through the capillary tube and mixing is initiated. Next, the piston part 7 is raised relative to the cylinder part as illustrated in Fig. 7, thereby sucking the combined volumes of sample and diluting liquid through the space between the bore and the capillary tube and into the lower portion of the cylinder part, i.e., the primary cylinder portion 15 forming also a second mixing chamber.

It is evident that the preferably annular space between the capillary tube and the bore, i.e., the relative dimensions of the capillary tube and the bore, must be adapted to one another, and to the viscosity of the fluids, such that a sufficient part of the flow passes through the capillary tube

during the first downward stroke to displace all the sample contained therein.

The reciprocating movement is repeated a predetermined number of times sufficient for performing a thorough mixing of the sample and the diluting liquid, thereby providing a diluted sample DS.

It is now preferred to make a pause, typically for 45 seconds, and then to make a blank measurement of certain parameters of the diluted sample DS contained in the primary portion 15 of the cylinder part 8 in order to obtain a reference value. Such measurement is performed here by means of a light path 18 through the cylinder part 8 enabling photometric determination of the desired parameters. The light path extends between a light source 19 and a light sensor 20.

After having obtained the desired reference value, the piston part 7 is caused to perform an extended, or, full downward stroke as indicated in Fig. 8. Hereby, the lower end of the capillary tube 1 penetrates the diaphragm 17 as likewise indicated in Fig. 8. It will be seen, particularly from a comparison of Fig. 6, showing the lower end 3 of the capillary tube approaching the diaphragm 17 at the end of its limited downward stroke, and Fig. 8, showing the piston part 7 at the end of its full downward stroke, that displacement between these positions will cause the diluted sample DS to be pressed through the ruptured diaphragm into the secondary space 16 and bring along antibodies A up through the capillary tube and into the widened portion 12 of the bore 10. Naturally, some flow of diluted sample also occurs between the capillary tube and the lower portion 11 of the bore.

A subsequent full upward stroke of the piston part 7 as shown in Fig. 9 will cause the diluted sample DS and the antibodies A contained therein to be drawn from the widened portion 12 between the capillary tube and the lower portion 11 of the bore into the lower portion of the cylinder part. This reciprocating operation is performed a predetermined number of times sufficient for performing a thorough mixing of the diluted sample DS and the antibodies A, thereby providing a mixture of diluted sample and antibodies DSA.

It is now preferred to make a pause, typically for 120 seconds, and then to make a photometric measurement by means of the light path 18. When making a CRP test, the photometric measurement takes place at typically 654 and 950 nanometers.

A second, preferred embodiment of the present invention is shown in Figs. 10 and 11 and is particularly intended for use with an analysis instrument as shown in Fig. 12. Where equal or similar elements are used as in the first embodiment, the same reference numerals will be used. It will be noted that neither sample nor diluting liquid or antibodies are shown in Figs. 10 and 11.

As in the first embodiment, the device includes a piston part 7 and a cylinder part 8, the piston part having an axial bore 10 having a lower portion 11 and an upper, widened portion 12.

A modified combined handle and cover 21 carries the capillary tube 1 such that its upper end 2 opens in an aperture 22 extending through a lower portion 23 of the cover having a smaller outer diameter than the widened portion 12 of the bore 10. Thus, in the position shown, the aperture 22 communicates with the bore 10 through the annular space between the cover portion 23 and the widened bore portion 12. An intermediate

portion 24 of the cover fits within a further widened portion 25 of the bore 10 and a sealing ring 26 seals between the cover and the bore portion 25. The axial position of the capillary tube is defined by abutment between shoulders 27, 28 of the cover and the piston part, respectively.

In relation to the first embodiment, the piston part is downwardly extended such that the capillary tube is mechanically protected by not protruding below the lower face 29 of the piston part. Instead, the lower face 29 is provided with a downwardly directed protrusion 30 having a sharpened tip 31. The bore 10 has an extension 32 through the protrusion 30. This extension may have the same internal dimension as the bore portion 11 but is shown here to have a smaller cross section, so that the extension 32 is connected to the bore portion 11 by a downwardly tapering bore portion 33.

In order to protect the piston part from contaminating contact, and particularly its lower end including the tip 31 and the bore extension 32, the piston part is provided with a skirt 34 surrounding it and extending a substantial distance beyond its lower face 29. In the assembled state of the device shown, the skirt also surrounds the cylinder part 8 such that relative axial movement between the piston part and the cylinder part is possible. Since location of the cover 21 and the capillary tube in the position shown involves a certain degree of downward axial force upon the cover 21, and, thus, upon the piston part, the axial extension of the skirt 34 is such that it extends at least to and preferably somewhat beyond the lower end of the cylinder part in the preparatory state of the device as shown in Fig. 10. Consequently, when in practice the device is put on a support, e.g. a table, with the lower free end of the skirt supporting the device,

downward force applied on the cover cannot push the piston part any further into the cylinder part.

The relative positions of the parts shown in Fig. 10 correspond to those shown in Fig. 5, i.e., before a downward stroke of the piston part. Such stroke will force diluting liquid contained in the primary cylinder portion 15 below the piston part up through the bore extension 32. From there one part of the liquid will enter the capillary tube 1 to displace sample contained therein. Another part of the liquid will flow between the capillary tube and the bore portion 11 to enter the widened bore portion 12 and mix there with the sample and the part of diluting liquid forced through the capillary tube into the aperture 22.

The relative positions of the parts shown in Fig. 10 also correspond to those shown in Fig. 7, i.e., after an upward stroke of the piston part performed to draw the mixture contained in the bore 10 down into the primary cylinder portion 15.

Fig. 11 shows the device after the piston part 7 has performed a full downward stroke in relation to the cylinder part 8 and the sharpened tip 31 of the protrusion 30 has penetrated the diaphragm 17. This position corresponds to the position shown in Fig. 8, i.e., substantially the entire volume of diluted sample has been forced into the bore 10. A subsequent full upward stroke of the piston part will force that volume back into the primary cylinder portion 15. At least at the beginning of such stroke, a jet of liquid will flow through the ruptured diaphragm 17 and flush the secondary space 16, thereby ensuring a thorough mixing of the material contained therein with the diluted sample.

As already mentioned, the embodiment of Figs. 10 and 11 is particularly adapted for use with an analysis instrument 35 as shown in Fig. 12. The instrument includes a housing 36 having in a wall 37 thereof a recess 38 adapted to receive the device 6. Thus, the width of the recess is adapted to the external width of the device 6, i.e., its piston part 7 including the skirt 34. A fixed pin 39 protrudes centrally from a lower part of an inner wall 40 of the recess, and two spaced operating arms 41, 42 protrude through vertically directed apertures 43, 44 in the inner wall 40 so as to be simultaneously movable limited distances up an down along opposed inner walls 45, 46 of the recess 38 as indicated by arrows.

As appears from Figs. 10 and 11, an upper portion of the piston part 7 is provided with engagement means in the shape of opposed recesses 47, 48 spaced and dimensioned so as to be engaged by a respective operating arm 41, 42 when the device 6 is located in the recess 38. A hole 49 is provided through a lower portion of the cylinder part 8 and is adapted to engage the fixed pin 39 when the device is located in the recess.

Control means and actuating means (not shown) are adapted to move the arms 41, 42 upwards and downwards through strokes that are preferably adjustable regarding number, amplitude and frequency.

The instrument 35 also includes a light path 18 having a light source 19 and a light sensor 20. In order to enable light to pass into and through the primary cylinder 15, as indicated by the light path 18 in Fig. 10, opposite holes 50, 51 are provided in the skirt 34.

An initial position of the operating arms 41, 42 in relation to the fixed pin 39 is that corresponding to the relative

positions of the piston part 7 and the cylinder part 8 shown in Fig. 10. In use, a device 6 having a capillary tube 1 charged with a blood sample is located in the recess such that the hole 49 engages the fixed pin 39 and the recesses 47, 48 engage the arms 41, 42, respectively. In that position, movement of the arms 41, 42 will impart a corresponding movement to the piston part, while the cylinder part is kept stationary by the pin 39 engaging the hole 49. As seen, particularly in Fig. 11, the skirt 34 is provided with at least one slit 52 enabling movement of the piston part relative to the stationary cylinder part.

A programmed evaluating sequence is started, either automatically a predetermined time after location of the device in the recess 38, or, manually by an operator by pressing an appropriate button (not shown). Such sequence may include the steps referred to during the discussion of the embodiment shown in Figs. 5 - 9, i.e., a first diluting and mixing step involving limited downward and upward piston strokes, a pause, a blank measurement, a second mixing step involving a first full downward piston stroke causing piercing of the diaphragm 17 and subsequent full upward and downward strokes to obtain further mixing, a pause, and, finally, one or more measurements by means of the light path 18. The results obtained will be exhibited on a display 53.

CLAIMS

1. A device for diluting and mixing a liquid sample, said device including receiving means for a tubular sample recipient, first receptacle means for a diluting liquid, and mixing chamber means, c h a r a c t e r i z e d by

- a piston part (7) having a forward end and a backward end, an axial bore (10) through said piston part defining said receiving means and defining a first mixing chamber (12) located at said backward end; and
- a cylinder part (8) having an open end and a closed end, said cylinder part defining said first receptacle means (15), and said first receptacle means constituting also a second mixing chamber;

said piston part (7) being introduced through said open end to be sealingly and slidingly movable within said cylinder part (8), whereby relative movement of said piston part (7) towards said closed end causes diluting liquid (D) contained in said first receptacle means (15) to flow through said bore towards said first mixing chamber (12) thereby at least partly flushing through a tubular sample recipient (1) positioned in said bore (10) to mix in said first mixing chamber (12) with a sample (S) displaced from said tubular sample recipient to form a diluted sample (DS), and whereby relative movement of said piston part (7) away from said closed end causes flow of said diluted sample through said bore towards said second mixing chamber (15).

2. A device according to claim 1, c h a r a c t e r i z e d in that said bore (10) has a greater diameter than said tubular sample recipient (1) to allow fluid flow between said bore and said tubular sample recipient.

3. A device according to claim 2, c h a r a c t e r i z e d in that said first mixing chamber comprises a widened portion (12) of said bore (10).

4. A device according to claim 1, c h a r a c t e r i z e d in that said cylinder part defines a second receptacle means (16) located at said closed end and separated from said first receptacle means by diaphragm means (17).

5. A device according to claim 4, c h a r a c t e r i z e d by piercing means (3; 31) movable with said piston part (7) to pierce said diaphragm means (17) upon movement of said piston part towards said closed end.

6. A device according to claim 5, c h a r a c t e r i z e d in that said piercing means (31) is hollow and communicating with said bore (10).

7. A device according to claim 1, c h a r a c t e r i z e d in that said cylinder part is at least partly translucent.

8. A device according to claim 3, c h a r a c t e r i z e d in that said widened portion (12) includes a portion (25) adapted to receive a holder (21) carrying said tubular sample recipient.

9. A device according to claim 1, c h a r a c t e r i z e d in that said piston part (7) includes support means (34) adapted to prevent undue movement of said piston part (7) into said cylinder part (8).

10. A device according to claim 1, c h a r a c t e r i z e d in that said piston part (7) and said cylinder part (8) include respective engagement means (47, 48, 49) adapted for

positive engagement by movable engagement means (41, 42) and fixed engagement means (39), respectively.

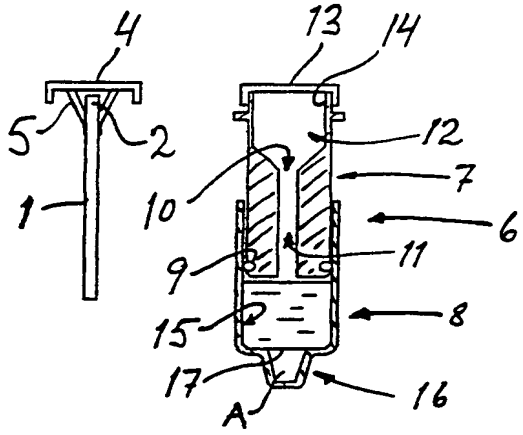


Fig. 1 Fig. 2

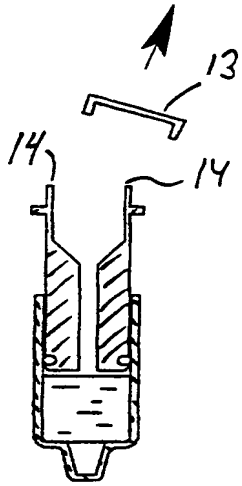


Fig. 3

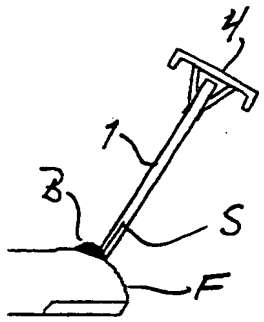


Fig. 4

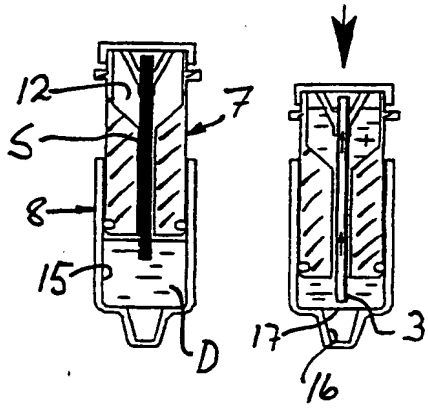


Fig. 5 Fig. 6

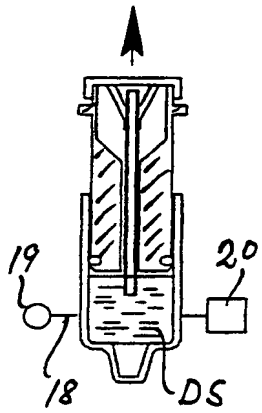


Fig. 7

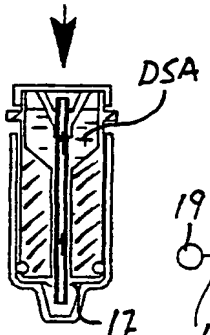


Fig. 8

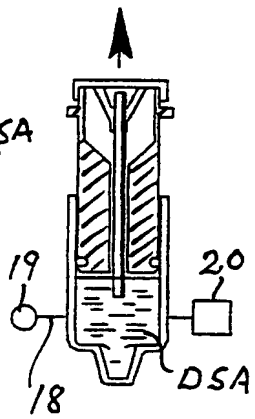


Fig. 9

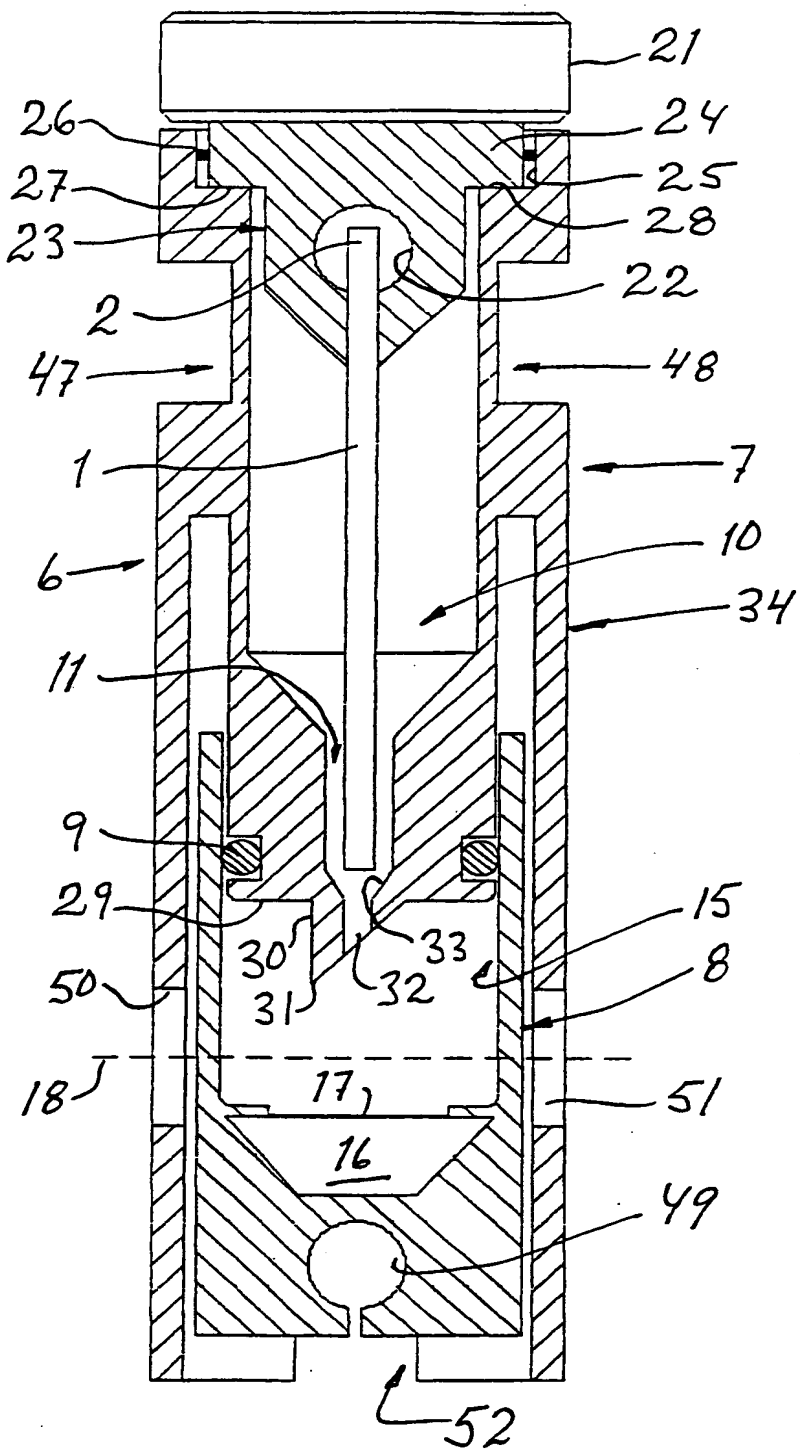

21
26
24
27
25
23
28
22
2
47
48
1
7
6
10
34
11
9
29
15
30
33
31
32
50
8
18
17
51
16
49
52

Fig. 10

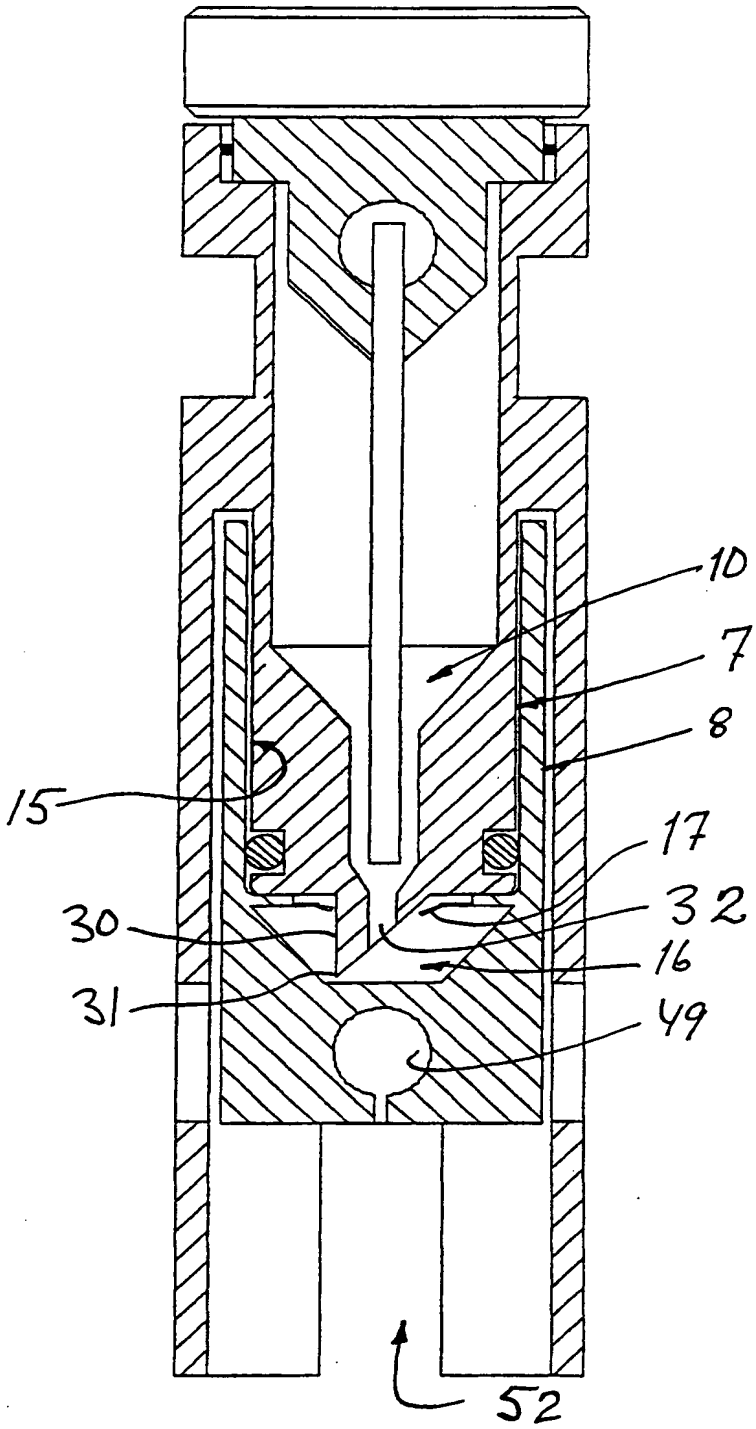


Fig. 11

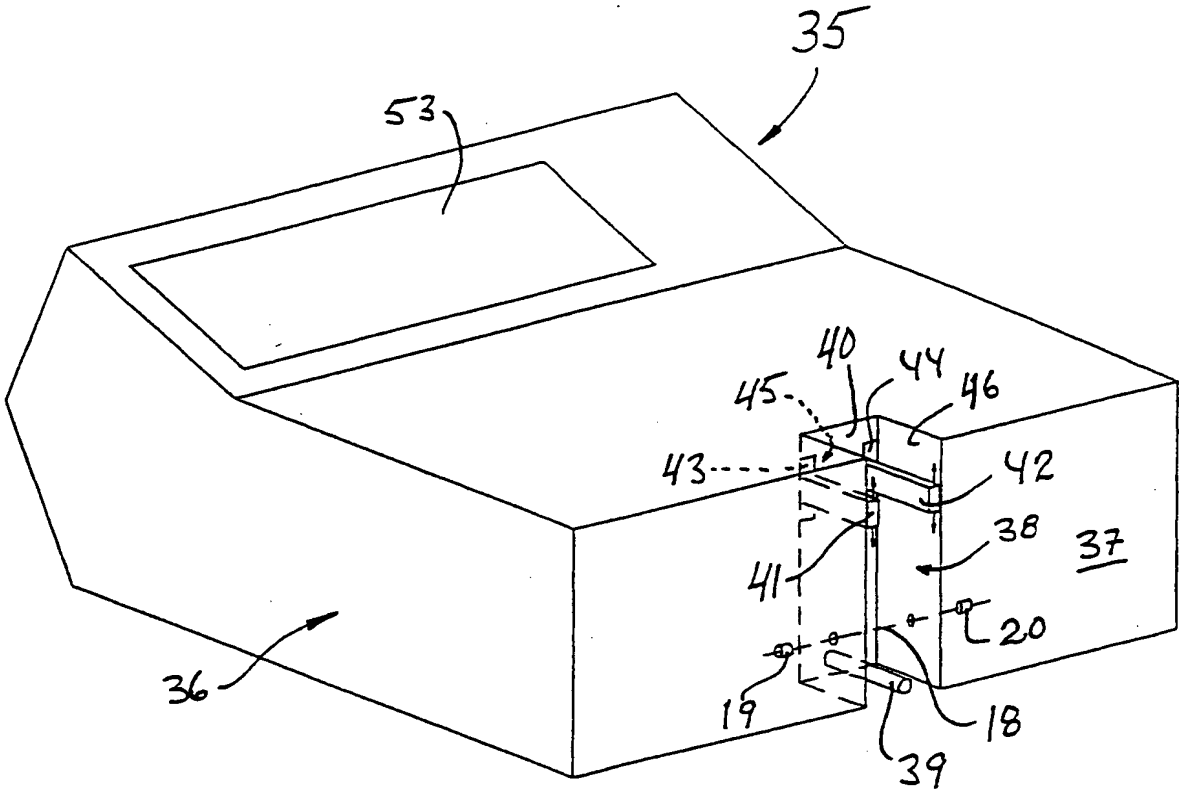


Fig. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/00705

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 1/38
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9822797 A1 (MEDONIC AB), 28 May 1998 (28.05.98), page 2, line 21 - page 3, line 12; page 6, line 18 - line 32	1-10
	--	
A	JP 9113421 A (KUNIMUNE KOGYOSHO:KK) 1997-09-30 (abstract) (online) (retrieved on 2001-06-20). Retrieved from: EPO PAJ Database.	1-10
	--	

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2001	05 -07- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Ulla Granlund/MP Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/01

Internal application No.
PCT/SE 01/00705

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9822797 A1	28/05/98	EP 0890090 A	13/01/99
		SE 507956 C	03/08/98
		SE 9604259 A	21/05/98
		US 6098471 A	08/08/00